Markus Hildinger (Customer Number 37439)
Phone: +39-333-8195052

Application Number 10/604,340

This communication is in reference to

Patent application number	10/604,340
Confirmation number	1339
Reference number	2
Examiner	Jon Benjamin Ashen
Art unit	1635
Invention title	DECREASING GENE EXPRESSION IN A MAMMALIAN SUBJECT IN VIVO VIA AAV-MEDIATED RNAi EXPRESSION CASSETTE TRANSFER
First inventor	Dr. Markus Hildinger
Second inventor	Dr. Alberto Auricchio
Filer	Dr. Markus Hildinger Cranachweg 8 75173 Pforzheim; Germany Phone: +39-333-8195052 E-mail: hildinger@gmx.net Customer number: 37439.

It comprises two parts:

Part I	Response to Office Action Summary from 6/17/2005 including request for amending the claims (A) and specification (B) of patent application 10/604,340
Part II	Paper copy of sequence listing

Markus Hildinger
Signed

July 14th 2005
Date

Included

(1) Request for amending the claims and specification

(2) Paper copy of sequence listing

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REQUEST FOR AMENDING PATENT APPLICATION 10/604,340
Part I of Communication

This is a request – in compliance with Chapter 714 (III. "Revised manner of making amendments") of the MPEP and 37 CFR 1.121 – for amending

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First inventor	Dr. Markus Hildinger
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Filer	Dr. Markus Hildinger Cranchweg 8 75173 Pforzheim Germany Phone: +39-333-8195052 E-mail: hildinger@gmx.net Registration number: 37439.

This amendment does not go beyond the disclosure of the application as originally filed (i.e., contains no new matter). It primarily addresses compliance with the sequence rules.

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Signed

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(1) Amendment to Claims (11 claims amended): 8 pages

(2) Amendment to Specifications (18 amendments): 6 pages

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Response to Office Action Summary from 6/17/2005

The inventor first would like to thank the examiner for the helpful discussion of the present patent application over the phone. The inventor also allows the examiner to use e-mail as a means of communication.

Response to: Sequence Compliance

The author electronically submitted a sequence listing on 7/10/2003 as well as a paper copy of the electronic sequence listing (EFS ID 48969).

However, the author inadvertently did not reference (i.e., "SEQ ID NO: xxx") the sequences within the specification. Thus, the author amends the specification with this communication to add the sequence references in order to comply with 37 CFR 1.821(d).

Furthermore, when compiling the sequence listing, the author inadvertently did not include the sequence for AAV 2/5 U6/U6 lucRI-2. However, the sequence (together with all other sequences) was originally submitted as part of the specification. Thus, no new subject matter will be added. To comply with 37 CFR 1.821 – 1.825, the author – as part of this communication – submits a substitute paper copy of the computer readable form, which now includes the sequence listing for AAV 2/5 U6/U6 lucRI-2 as SEQ ID NO:12. The substitute electronic version (CRF) has been submitted on July 13th 2005 (EFS ID 88046).

Response to: Election/ Restrictions

Response to <1>

The inventor agrees with the examiner's view and selects group I, drawn to an in vivo method of decreasing the expression of a target gene in a mammalian cell.

Response to <2>

see response to <1>

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Response to <3> and <4>

The inventor selects group c ("short hairpin RNAs"), but traverses the examiner's view, i.e., the inventor considers groups a, b, and c not patentably distinct.

The RNA molecules expressed under groups a, b, and c – although different in sequence – will perform the same biologic action and function, i.e., they will become a substrate (after processing by Dicer) for the RNA-induced silencing complex (RISC; for review, see Zamore, P. D., RNA interference: listening to the sound of silence. Nat Struct Biol, 2001. 8(9): p. 746-50.; McManus, M. T. and P. A. Sharp, Gene silencing in mammals by small interfering RNAs. Nat Rev Genet, 2002. 3(10): p. 737-47; Hammond, S. M., A. A. Caudy, and G. J. Hannon, Post-transcriptional gene silencing by double-stranded RNA. Nat Rev Genet, 2001. 2(2): p. 110-9.) For example, Zamore teaches us in the afore-mentioned Nat Struct Biol paper that RNA interference only depends on the presence of double stranded RNA, which is procssessed by the cell into 21 to 23 nucleotides of siRNA, which then forms an siRNP complex that will become part of the RISC complex. Thus, the RNA molecules are functionally and biologically not distinct as they will all become part of the RISC complex to mediate RNA interference.

Furthermore, the RNA molecules are structurally and chemically identical as they are all made up of ribonucleotides with the bases uracil, guanine, cytosine and adenine in the end. The inventor acknowledges that the RNA molecules might have different lengths and base compositions, but – in the eye of the inventor – this would not make them patentably distinct in the spirit of the present invention.

Last, the present invention claims and demonstrates downregulation of gene expression in vivo via RNA interference using adeno-associated viral vectors to express RNAi expression cassettes. Thus, all designs or strategies that can lead to RNA interference should be considered. The inventor also would like to point out that the RNAi expression cassette design for species a, b, and c is already in the public domain, e.g.,

- for species a (one short interfering RNA – dual promoter): Lee, N. S., et al., Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nat Biotechnol, 2002. 20(5):p. 500-5;
- for species b (two separately transfected constructs): Lee, N. S., et al., Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nat Biotechnol, 2002. 20(5):p. 500-5; with a comparison between species a and b in figure2;

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- for species c (short hairpin RNA): Brummelkamp, T. R., R. Bernards, and R. Agami, A system for stable expression of short interfering RNAs in mammalian cells. Science, 2002. 296(5567): p. 550-3.

However – at the time of filing – it has not yet been demonstrated that RNA interference can be achieved in vivo by using AAV-mediated gene transfer.

The inventor believes it will not be a serious burden on the examiner to perform the search on all the species at once; it actually might be more difficult to identify prior art by only searching for the species a, b, and c rather than by searching for, e.g., "RNA interference" AND "AAV". For example, the inventor performed a PubMed search for RNA Interference with AAV (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=search&term=AAV+RNA+interference) on 7/12/2005, and only five entries were returned with the earliest entry dating back to 2004. For comparison, entering the search term hairpin and AAV, one will retrieve many documents unrelated to the subject matter, but related to the ITR-hairpin structure of AAV.

Thus, the inventor kindly asks the examiner to reconsider his request for restriction.

Response to <5>

The inventor agrees with the examiner's view and amends claim 36 in the present communication accordingly.

Response to <6>

The author appreciates the examiner's comments in respect to the claim linkage and thanks the examiner.

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Response to <7>

The inventor selects "pol III", yet traverses the examiner's view, i.e., the inventor does not agree with the examiner's consideration for restriction and considers the use of different promoters not patentably distinct.

The species are obvious variants, and it is known in prior art that the transcription of a genetic sequence can be achieved through a variety of promoters. In deed, it has been shown that RNA interference can be achieved through the use of pol II as well as pol III promoters, e.g.,

- For pol III promoters: Brummelkamp, T. R., R. Bernards, and R. Agami, A system for stable expression of short interfering RNAs in mammalian cells. Science, 2002. 296(5567): p. 550-3;
- For pol II promoters: see U.S. patent application 20040023390 (Davidson et al.). This patent application is – in the eyes of the inventor – extremely relevant as prior art document as it relates to viral vector mediated RNA interference with pol II promoters. (The inventor was unaware of this patent application at the time of filing his own application, which – of course – still makes patent application 20040023390 prior art.)

To the best knowledge of the inventors, at the time of filing, it has not yet been reported that RNA interference can be achieved with pol I driven RNAi expression cassettes.

The inventor believes it will not be a serious burden on the examiner to perform the search on all three promoters. Furthermore, the present invention claims and demonstrates downregulation of gene expression in vivo via RNA interference using adeno-associated viral vectors to express RNAi expression cassettes. Thus, the choice of promoter should not be seen as a limitation or restriction on the present invention, i.e., even promoters other than pol I, II, or III should be within the scope of the invention.

Response to <8>

See election under response to <1>